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Abstract: Parvovirus 4 (PARV4) is a DNA virus frequently associated with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections, but its clinical significance is unknown. We studied the prevalence of PARV4 antibodies in 2 cohorts of HIV- and HCV-infected individuals (n = 469) and the correlations with disease status. We found that PARV4 infection frequently occurred in individuals exposed to bloodborne viruses (95% in HCV-HIV coinfecting intravenous drug users [IDUs]). There were no correlations between PARV4 serostatus and HCV outcomes. There was, however, a significant association with early HIV-related symptoms, although because this was tightly linked to both HCV status and clinical group (IDU), the specific role of PARV4 is not yet clear.

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Parvovirus 4 Infection and Clinical Outcome in High-Risk Populations

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Parvovirus 4 (PARV4) is a DNA virus frequently associated with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections, but its clinical significance is unknown. We studied the prevalence of PARV4 antibodies in 2 cohorts of HIV- and HCV-infected individuals (n = 469) and the correlations with disease status. We found that PARV4 infection frequently occurred in individuals exposed to bloodborne viruses (95% in HCV-HIV coinfecting intravenous drug users [IDUs]). There were no correlations between PARV4 serostatus and HCV outcomes. There was, however, a significant association with early HIV-related symptoms, although because this was tightly linked to both HCV status and clinical group (IDU), the specific role of PARV4 is not yet clear.

Parvovirus 4 (PARV4) is a recently discovered DNA virus of the *Parvoviridae* family that infects human populations worldwide [1–5]. It has been frequently found in intravenous drug

users (IDUs) [4, 6] but is uncommon in healthy individuals in Western countries, suggesting parenteral transmission [4, 6].

Parvovirus 4 is associated with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections [3]; hence we were interested in the effect PARV4 might have on disease progression. Parvovirus 4 has been found in many tissues, including the bone marrow of HIV-positive individuals [3] and the liver of HCV-positive individuals [1, 7], but is also found in plasma, cerebrospinal fluid, skin, and myocardium [1, 2, 4, 6, 8]. Despite the detection of viral DNA in so many organs, PARV4 has rarely been linked to specific symptoms, except for a recent study linking PARV4 to encephalitis [8] and in the individual in whom PARV4 was discovered, who presented symptoms including pharyngitis, vomiting, and arthralgias [2]. Determining a pathological association is problematic because PARV4 is so frequently found in individuals burdened with HCV- or HIV-related disease. However, as a common coinfection with these pathogens, it may have an important impact on clinical disease. This is of increased importance in light of the recent examination of immune responses to this virus [9]. This revealed sustained high-frequency T-cell responses associated with an effector memory phenotype consistent with persistent exposure to immunogenic viral antigen. Because the antigen can be expressed in both lymphoid and hepatic tissues [3, 7], an impact on both HCV and HIV pathogenesis is plausible.

We therefore studied the impact of PARV4 serostatus on HCV- and HIV-related disease progression. To do this, we tested for PARV4-specific antibodies in 2 prospective cohorts of HCV- and HIV-infected individuals (mono- and coinfecting). Hepatitis C viral clearance, genotype, patient gender, age, alanine amino-transferase (ALT) levels, tissue histology (fibrosis), CD4 slopes, and Centers for Disease Control and Prevention (CDC) events were analyzed in relation to PARV4 serostatus.

METHODS

Study Subjects and Sample Collection

Local ethical approval (MREC/98/3/55 and <http://www.shcs.ch/30-study-design>) and informed patient consent were obtained for 2 cohorts, totaling 469 individuals (Table 1). The first, which consisted of 193 individuals from the Swiss HIV cohort [10], was made up of 99 HIV-positive, HCV-negative men who have sex with men (MSM) and 94 HIV-HCV coinfecting IDUs. The first sample taken at least 1 year after the first positive HIV serology was used in experiments. The

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Table 1. Demographic Data and Parvovirus 4 (PARV4) Seroprevalence

Cohort	Main Risk Factor	No.	HCV	HIV	Gender, % Male	HCV Genotype at Diagnosis	PARV4 Serostatus
Swiss HIV							
	MSM	99	–	+	100%	N/A	11%
	IDUs	94	+	+	74%	60% genotype 1, 26% genotype 3	95%
Nottingham Trent							
	66% IDUs	276	+	–	67%	40% genotype 1, 48% genotype 3	31%

Only 58 of 94 hepatitis C virus (HCV) genotypes for the Swiss HIV cohort were available. The percentages shown represent proportions of known genotypes. Abbreviations: HIV, human immunodeficiency virus; IDUs, intravenous drug users; MSM, men who have sex with men; N/A, not applicable.

second group, which consisted of 276 individuals from the Trent HCV cohort, was divided into 44 individuals who cleared HCV vs 232 who became chronically infected and, among chronically infected individuals, 143 who had persistently mild liver fibrosis (Ishak fibrosis score always 0 or 1, 1–5 biopsies were taken per individual, across 1–25 years) vs 98 who progressed to severe fibrosis (Ishak fibrosis score 5 or 6). The most recent plasma sample available was tested in this latter cohort. Plasma samples were taken by each center and frozen until required.

Serological Screening

Plasma samples from these individuals were tested in duplicate for anti-PARV4 immunoglobulin G (IgG), as described elsewhere [6].

CD4 Slopes and CDC Events

CD4 counts were available for 114 of 193 individuals of the Swiss HIV cohort prior to antiretroviral therapy. CD4 slopes were calculated using GraphPad Prism when at least 3 time points were available, spanning 6 months minimum. CD4 counts were plotted relative to time (day 0 was the first available sample date) to determine CD4 slopes, which were then compared to PARV4 serostatus using Mann–Whitney tests. Kaplan–Meier survival curves were drawn to compare time to reach CDC-B and CDC-C events and death, defined by the CDC as hallmarks of HIV-related disease [11].

Statistical Analyses

Statistical analyses were carried out using the Mann–Whitney test, Fisher’s exact test, and log rank (Mantel–Cox test) using GraphPad Prism software. For more detailed statistic analyses on the Trent HCV cohort, the open-source R software (<http://www.r-project.org>) was used to perform a logistic regression to analyze the simultaneous relationship of age, gender, main risk factor (IDU or MSM), and ALT with PARV4 serostatus. The HCV genotype was excluded because this information was not available for all individuals. The log odds of being PARV4 seropositive were modeled as a linear function of all

variables potentially affecting this. The formula was:

$$\text{Log odds (PARV4 serostatus)} \sim \text{age} \\ + \text{gender} + \text{main risk factor} + \text{ALT}$$

where “~” implies relationship. The coefficients of the model have approximately normal distributions; therefore *P* values were obtained to compare individual coefficients. This analysis was carried out on 263 individuals of the Trent HCV cohort with complete data sets.

RESULTS

PARV4 Strongly Correlates With Intravenous Drug Use and Is Linked to CDC-B Event Occurrence

To address whether PARV4 has an impact on HIV disease progression, PARV4 serostatus was determined in 193 individuals from the Swiss HIV cohort (Table 1). First, we noted a striking association between PARV4 status and clinical group. In the HIV-positive, HCV-positive (IDU) group, a 95% prevalence of PARV4 was observed, compared with only 11% in the HIV-positive MSM group (*P* < .0001). We next analyzed CD4 slopes in relation to PARV4 status using serial CD4 counts available for 114 patients: 49 PARV4 IgG[–] individuals with CD4 slopes ranging from –41.3 to 5 cells/μL/month (median, –6.2), and 65 PARV4 IgG⁺ individuals with CD4 slopes ranging from –32.6 to 7.6 CD4 cells/μL/month (median, –6.3; data not shown). Using this approach we could not detect a difference in CD4 T cell decline between serologic groups (*P* = .89).

As another robust indicator of HIV disease progression, we assessed the occurrence of CDC-B and CDC-C events and death in PARV4-seropositive and -seronegative groups (Figure 1). The CDC-B symptoms were observed earlier in PARV4-seropositive individuals (*P* = 0.02). The association was not significant for CDC-C or death or between clinical groups (MSM vs IDU; data not shown). Thus, we did observe a correlation between PARV4 serostatus and early HIV disease progression, but the very close association between PARV4 serostatus, risk factor for HIV

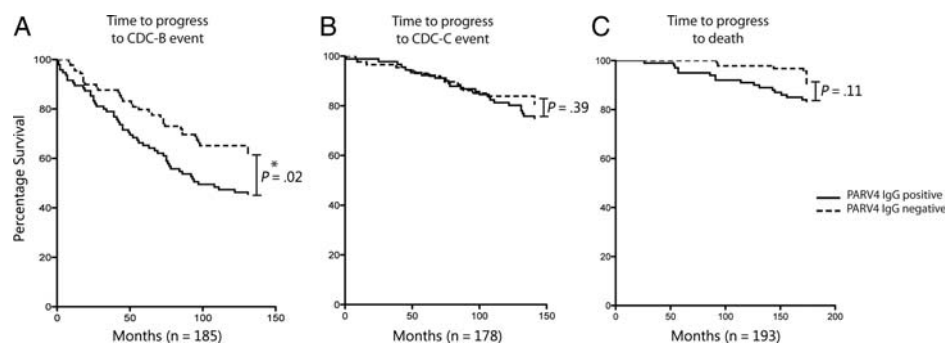


Figure 1. Time to progress to Centers for Disease Control and Prevention (CDC)-B events (A), CDC-C events (B), and death (C) in each parvovirus 4 (PARV4) serology group. All individuals from the Swiss HIV cohort were grouped for this study. Time is expressed in months since human immunodeficiency virus (HIV) seroconversion. Each survival curve was compared using log-rank (Mantel-Cox test), and *P* values are indicated adjacent to each graph. * represents statistical significance. A significant difference was observed between PARV4 immunoglobulin G (IgG) positive and IgG[−] in the time to progress to CDC-B events.

transmission, and HCV serostatus makes the individual components of risk difficult to dissect.

PARV4 Infection Is Also Common in HCV Mono-Infected Individuals

The prevalence of PARV4 was also studied in 276 HCV-positive, HIV-negative individuals from the Trent HCV cohort. Thirty-one percent of individuals were PARV4 seropositive, and 36% in the IDU subset were PARV4 seropositive.

To determine if PARV4 serostatus affects clinical outcome in HCV-positive individuals, age, gender, HCV genotype, ALT levels, and main risk factor (IDU or non-IDU) were compared. No significant difference was seen between PARV4-seropositive and -seronegative individuals in gender, HCV genotype, age, or ALT levels (*P* = .89, .64, .26, and .34 respectively). We did, however, find a strong association between PARV4 serostatus and intravenous drug use as a risk factor (*P* = .01).

Next, the parameters of age, gender, ALT, and risk factor were analyzed together with PARV4 serology using a logistic regression framework. The results of this analysis show that only the IDU risk group significantly correlated with detection of PARV4 IgG (*P* = .01).

PARV4 Does Not Affect the Progression of HCV-Related Disease

To assess the impact of PARV4 infection on HCV disease progression, 276 individuals from the Trent HCV cohort were grouped by clinical outcome. Study subjects were divided by PCR status: either persistently positive (*n* = 232) or always negative (*n* = 44), an indication of spontaneous HCV clearance. Thirty-six percent of individuals who had cleared HCV were PARV4 seropositive vs 29% of individuals who remained viremic (*P* = .37; data not shown).

Longitudinal biopsy data was available for 241 of these individuals. They were divided into those who maintained a mild

fibrosis score (Ishak score 0 or 1 over time; *n* = 143) and those who progressed to severe liver fibrosis (Ishak score 5 or 6; *n* = 98). Twenty-nine percent of the individuals with mild fibrosis were PARV4 seropositive vs 31% of those with progressive disease (*P* = .78; data not shown). Finally, the treatment outcome of 131 individuals from the Trent HCV cohort was compared with their PARV4 serostatus. No significant difference was seen between patients who did not respond to treatment or relapsed and those who had a sustained virological response (*P* = .45; data not shown).

DISCUSSION

This study confirms that PARV4 infection is common in HCV-positive individuals, and even more so in HCV-positive IDUs coinfecting with HIV. Although PARV4 did not influence HCV persistence, disease progression, or treatment response, PARV4-seropositive HIV-infected subjects progressed faster to HIV CDC-B symptoms than PARV4-seronegative individuals. However, because this was tightly linked to both HCV status and clinical group (IDU), the specific role of PARV4 is not yet clear.

Thirty-one percent of the HCV-positive, HIV-negative individuals of the Trent HCV cohort were PARV4 seropositive. This is in accordance with our previous study that found 26% seropositivity in 84 HCV-infected individuals [9] and other studies that tested for PARV4 IgG [6] or viral DNA directly [12]. Parvovirus 4 infections are acute and resolve rapidly, followed by complete or almost complete clearance of viremia and only very low viral loads on reactivation [12]. Parvovirus 4 may therefore be relatively inefficiently transmitted compared with HCV and HIV-1.

In the Swiss HIV cohort, a striking 95% of the HIV-positive, HCV-positive IDUs were PARV4 seropositive. This is the

highest published figure for this clinical group, with other studies reporting 55%–85% [6]. Together with only 11% PARV4 seropositivity of the HIV-positive, HCV-negative MSM and 31% in the Trent HCV cohort, these data strongly indicate parenteral transmission of PARV4. Parvovirus 4 seroprevalence in HCV-positive, HIV-negative IDUs was still only 36%. This suggests that HIV infection in the Swiss cohort may be a marker for a more highly exposed IDU subset, although other factors such as differences in risk behavior, local epidemiology, and also an impact of HIV infection itself may potentially play a role. Parvovirus 4–specific antibodies were also present in non-IDU MSM, albeit in fewer individuals, which suggests unreported intravenous drug use or another transmission route because all these individuals were also HCV negative. The possibility of alternative routes of transmission is strengthened by studies in HIV-positive MSM [6], along with reports of the presence of PARV4 in the general population in Central Africa and in children in India [5, 8].

The study cohorts were not further analyzed for the rate of PARV4 seroconversion. These are prospectively clinically monitored groups, and the exposure rates do not necessarily reflect an active IDU status. Further studies to define the incidence of new PARV4 infections in active IDU cohorts are underway, and these will help define the duration of viremia and the timing of acquisition of antibody and T-cell responses.

These 2 cohorts have allowed us to study the relationship between PARV4 serostatus and age, gender, ALT levels, HCV genotype, HCV treatment outcome, and HCV or HIV disease progression. Although we found no correlation between PARV4 seropositivity and HCV disease, significant correlations were found between PARV4 and early HIV disease–related symptoms. However, this clear association is tightly linked to both HCV status and clinical group (IDU). Although there is clear evidence that PARV4 DNA persists in tissues, we did not test for viral DNA because PARV4 viremia is rarely detected [3].

Immunosuppression from HIV infection is known to accelerate HCV disease progression, linked to increased HCV loads and local immune activation in the liver [13, 14]. In the case of increased mortality in HCV-positive, HIV-positive cohorts, it is clearly hard to distinguish the impact of HCV infection per se and the impact of the associated risk factor of intravenous drug use or even another coinfection such as PARV4. The IDU profile may also affect subject compliance to treatment, which may allow the disease to progress faster.

The factors that determine HCV disease progression in those chronically infected are more complex, although they do include environmental factors such as alcohol use in addition to gender and age. Although PARV4 infection did not have a significant impact in our relatively small, although well-defined cohort, larger prospective studies could address this further.

PARV4 remains an intriguing virus that is not associated with clear symptoms. Further research on the pathogenesis of

PARV4 infection, especially acute infection, is warranted given the extremely high prevalence of infection in HIV-positive, HCV-positive IDUs and the profound and long-lived T-cell responses elicited. Future studies to define its cellular and tissue targets may well shed further light on its pathogenesis, both as a single agent and in the context of coinfection.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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